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I U C L I D

D a t a S e t

Existing Chemical	ID: 68953-84-4
CAS No.	68953-84-4
EINECS Name	1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs.
EINECS No.	273-227-8

Producer Related Part
Company: ACC Rubber and Plastics Additives Panel
Creation date: 31-July-2000

Substance Related Part
Company: ACC Rubber and Plastics Additives Panel
Creation date: 31-July-2000

Printing date:	22-JAN-2003
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Reliability (profile): Reliability: without reliability, 1, 2, 3, 4

Flags (profile): Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. General Information ID: 68953-84-4

1.1 General Substance Information

Substance Type:
Physical Status: solid
Purity: 90 - 95 wt. %
Result: Molecular Weight: 274 (avg.)

1.1.1 Spectra

1.2 Synonyms

1,4-Benzenediamine, N,N'-mixed Ph and tolyl derivs.

Accinox 100

Blend of phenyl and tolyl p-phenylenediamines

DAPD

Mixed diaryl-p-phenylenediamines

Mixed di-aryl-p-phenylenediamines

Diaryl-p-phenylenediamines

Naugard 496

Vulkanox 3100

Wingstay 100

Polystay 100

WTR Number 4a

Nailax (Nailax B)

Remark: Complex reaction product containing;
N,N'-di(o-tolyl)-p-phenylenediamine;
N.N'-Diphenyl-p-phenylenediamine; and
N-Phenyl-N'-(o-tolyl)-p-phenylenediamine

1.3 Impurities

CAS Number: 95-53-4
EINECS Number: 202-429-0
Chemical Name: o-Toluidine
Contents: < 0.1 wt %

CAS Number: 62-53-3
EINECS Number: 200-539-3
Chemical Name: aniline
Contents: < 0.1 wt %

CAS Number: 552-82-9
EINECS Number: 209-023-2
Chemical Name: Methyldiphenylamine
Contents: < 0.1 wt %

CAS Number: 122-39-4
EINECS Number: 204-539-4
Chemical Name: Diphenylamine
Contents: 1 - 5 wt %

1.4 Additives

2.1 Melting Point

Value:	90 - 105 degree C
Decomposition:	ambiguous
Method:	other: ASTM D-1519
Year:	1993
GLP:	no
Reliability:	(2) valid with restrictions Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.

31-JUL-2000 (35)

2.2 Boiling Point

2.3 Density

Type:	
Value:	
Method:	Other: ASTM D-891
Result:	Specific Gravity: 1.18
Reliability:	2) valid with restrictions Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.

31-Jul-2000 (34)

2.4 Vapour Pressure

2.5 Partition Coefficient

log Pow:	3.4 - 4.3
Method:	OECD Guide-line 117 "Partition Coefficient n-Octanol/Water), HPLC Method"
Year:	1995
GLP:	yes
Remark:	The product exhibits much lower values than DDT (6.2) which provides a benchmark for highly bioaccumulative chemicals. The test substance contains 3 major components.
Result:	# Methyl Groups -0 log Pow 3.37 # Methyl Groups -1 log Pow 3.82 # Methyl Groups -2 log Pow 4.28 The major components of the test substance displayed partition coefficients between 3.4 and 4.3.[as prescribed by 1.1-1.4 (Wingstay 100, mixed diaryl-p-phenylenediamines)]
Reliability:	(1) valid without restriction

01-AUG-2000 (29)

log Pow: > 3.7 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: for N,N'-Diphenyl-p-phenylenediamine
Reliability: (1) valid without restriction
20-FEB-2001 (9)

log Pow: > 4.3 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: For N-phenyl-N'-(o-tolyl)-p-phenylenediamine
Reliability: (1) valid without restriction
31-JUL-2000 (9)

log Pow: > 4.6 at 22.8 degree C
Method: other (measured)
Year: 1992
GLP: yes
Remark: For N,N'-Di(o-tolyl)-p-phenylenediamine
Reliability: (1) valid without restriction
20-FEB-2001 (9)

3.1.1 Photodegradation

3.1.2 Stability in Water

Type:
Method:
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)
Remark: See Biodegradation Studies
Reliability: (1) valid without restriction
31-JUL-2000 (23)

3.3.1 Transport between Environmental Compartments

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, domestic
Concentration: 100 mg/l related to Test substance
Degradation: .64 % after 28 day
Result: other: not readily biodegradable
Method: OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year: 1994 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)
Reliability: (1) valid without restriction
31-JUL-2000 (23)

Type: aerobic
Inoculum: activated sludge
Degradation: 0 % after 28 day
Method: other: OECD 301 Manometric Respirometry, modified according to EEC Round Robin Test "Assessment of Respirometry" DGX 1/283/82
Rev. 6, EEC Directive 79/831, Annex V, Part C
Year: 1990 GLP: yes
Test substance: as prescribed by 1.1 - 1.4 (Mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
31-JUL-2000 (6)

3.6 BOD5, COD or BOD5/COD Ratio

Method: other: unknown
Method: other: unknown

Result: ThOD: 3056 mg/g
Reliability: (4) not assignable

(6)

Method: other: unknown
Method: other: unknown

Result: ThOD: 2.555 mg/mg
Reliability: (4) not assignable

(23)

3.7 Bioaccumulation

Species:	Cyprinus carpio (Fish, fresh water)
Exposure period:	56 day
Concentration:	.05 mg/l
BCF:	< 5000
Elimination:	
Method:	other: MITI Method for Testing the Degree of Accumulation of Chemical Substances in Fish Bodies
Year:	1998 GLP: yes
Test substance:	as prescribed by 1.1 - 1.4 (Wingstay 100, mixed diaryl-p-Phenylenediamines)
Method:	The test substance had an assumed purity of 100%. A pilot toxicity test used orange-red killifish (Oryzias latipes) (10 fish per level) exposed the test substance for 48-hours in a semi-static system. Stock solutions were prepared by dissolving the test substance and HCO-40 (hydrogenated castor oil; 20 times the amount of the test substance) in tetrahydrofuran. Following evaporation of the tetrahydrofuran,ion-exchanged water was added to the mixture to prepare a 500 mg/L stock solution of the test substance. Carp (Cyprinus carpio)was used as the test species for the Bioconcentration study. Based on the 48-hours toxicity results and analytical detection, the test concentrations used were Level 1 (high exposure level)-0.05 mg/L and Level 2 (low exposure level)-0.005 mg/L. The test tanks were 100 L glass tanks. The test solution was entered into mix tanks at a flow rate of two(2) mL/minute for the stock solution and 1600 mL/minute for the dilution water. For controls, HCO-40 was dissolved with ion-exchanged water to give a 800 mg/L solution. The duration of exposure was for 8-weeks. Dissolved oxygen in the test tanks was measured twice a week. The concentrations of the test substance in water for both Levels were analyzed twice per week throughout the study. The concentrations of the test substance in fish at both Levels were analyzed during Week -1, -2, -4, -6 and –8 {two (2) fish per week}. Control fish were analyzed at the initiation {two (2) fish} and at termination {two (2) fish} of exposure. Additional fish were subjected to analysis on Days -1, -5, and -8 following cessation of exposure on Study Day-56 to assess depuration of test substance from fish tissues. All tissue and test water samples were analyzed using high performance liquid chromatography (HPLC).

Water levels were analyzed by loading large volumes on C18 Sep Pak mini-column, which was then eluted from column with Acetonitrile containing 0.1% Formic acid. The final volume of eluate was 5 mL. Test fish were analyzed by measuring weights, body lengths, chopping into pieces, and extracting with Acetonitrile. The mixture was centrifuged {7000xg. Five (5) minutes} and the supernatant was filtered with absorbent cotton to a volume of 100 mL. Two (2) separate samples were analyzed to assess Diphenylamine (DPA) and Diaryl p-phenylenediamine (diaryl-PPD) components (87% of complex)and to assess higher molecular weight components (13 % of complex). All recovery and blank tests were carried out in duplicate.

Remark: For DPA and DPPD compounds, methyl substitution increased bioaccumulation in carp, consistent with increasing log Po values. Substantial variation occurred at each time point due to use of data from a maximum of 2 fish. While this project provided substantial data, further work was needed to calculate BCFs according to western (OECD) concepts, and to apply appropriate statistics to these data so as to provide basis for interpretation.

To address this issue, a project was conducted by McLaren Hart entitled "Statistical Calculations of Data from a Bioaccumulation Study with WINGSTAY 100 in Carp", November 25,1998. The analysis employed Monte Carlo methods; the maximum BCF value (Pk 5) was 6600, and depuration data confirmed the attainment of tissue steady state levels of WINGSTAY 100 components within 3 weeks. Depuration was confirmed to be < 5 days for all components.
Orange-red killifish (Oryzias latipes) were used in the pilot toxicity test.

Result: Bioconcentration Test: The laboratory had difficulty maintaining nominal concentrations, possibly due to rapid uptake and metabolism by the fish and partioning to tank surfaces. The test concentrations ranged from 60 to 100% of the nominal values. The Bioconcentration Factors (BCFs) were calculated from individual data for fish at each time point and by using time-weighted averages for water concentrations. Since the test substance was a complex reaction product with numerous peaks, there was a high degree of variability in the fish data resulting in a large range of BCF values (20-221 for Peak 1; 128-659 for Peak 2; 269-2460 for Peak 3; 776-3640 for Peak 4; 2980-11300 for Peak 5). Depuration results for components indicated half-lives were below five (5) days for all components with the exception to one (1) estimate of 44-days for Peak 5. This inconsistent value appears to be suspect since it is much higher than the value of 4.7 days that was obtained for the same Peak in the other concentration. Also, the value is inconsistent with the trend Observed for half-lives for Peaks 1 through 4.

Bioconcentration Factors (BCFs) were calculated by using individual data points, including those prior to reaching steady-state. Estimates of steady-state through the use of Monte Carlo modeling improved the estimations of the BCFs. The bioaccumulation data and depuration data can be used together in performing analyses, particularity when the collected bioaccumulation data contained information on half-lifes(i.e., time to reach steady-state). The Monte Carlo "best estimates" for BCFs were < 5000 for all components except Peak 5 which had a BCF of approximately 7000.
Pilot Toxicity Test: The 48-hour LC50 result for the test substance in orange-red killifish was 17.2 mg/L. **Please note**: this concentration was achieved only through the use of a surfactant {Hydrogenated Castor Oil (HCO-40)}, and is far above the test substance solubility in water (approximately 2 mg/L). MITI guidelines recommend levels for Bioaccumulation testing to be at 1/1000 and 1/10,000 of the LC50 value. The lower value would have been below the quantitation range; thus, 0.005 and 0.05 mg/L were chosen.

Test condition: Two (2) test concentrations were used: Level 1 (high exposure level)-0.05 mg/L and Level 2 (low exposure level)-0.005 mg/L

Reliability: (1) valid without restriction

(10)

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type:	flow through
Species:	Cyprinus carpio (Fish, fresh water)
Exposure period:	14 day
Unit:	mg/l Analytical monitoring: yes
NOEC:	.28
LC50:	.43
Method:	OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed di-aryl-p-phenylenediamines)
Method:	Test water was generated by adding the test substance in acetone to a larger volume of water which was stirred, allowed to settle, and then siphoned to a stock solution holding tank. This stock solution was then metered into exposure tanks for the fish experiments. A range-finding trial exposed carp to nominal levels of 2.5, 5, 10, and 25 mg/L (ppm) of the test substance. Survival rates were up to 80% within the first 48 hours for the three (3) highest dose levels and the 2.5 mg/L induced no mortality in the first 48 hours although 90% deaths were seen through Day six (6). In the definitive phase, duplicate test tanks contained 10 carp each and the test substance nominal concentrations of 0, 0.1, 0.23, 0.51, 1.1, and 2.5 mg/L (ppm). Chemical analysis (HPLC) of the test substance in the test tanks on Days -0, -3, -7, and -14 showed that mean concentrations for the 14-day test period were 0.053, 0.12, 0.19, 0.28, and 0.67 mg/L (ppm). Fish densities were 0.35 g biomass/L flowing test solution per day. Tank volume turnover for the flow-through system was 6.5/day. Carp were monitored daily for mortality and signs of erratic swimming behavior for 14 days during exposure. Body weights and lengths were recorded for representative fish prior to study initiation, and on all test fish on Day 14. A LC50 value was then calculated.
Result:	Carp died only at the highest test substance concentration; 2/20 on Day-3, 7/20 on Day-7, and 20/20 by Day-14. Other findings at the 0.67 mg/L (ppm) level included darkened pigmentation on the fish (likely due to adsorption of the test chemical), lethargic swimming behavior, and loss of equilibrium. There were no test substance-related effects on body lengths or weights.
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
20-FEB-2001 (30)

Type: flow through
Species: Oncorhynchus mykiss (Fish, fresh water)
Exposure period: 14 day
Unit: mg/l Analytical monitoring: yes
NOEC: .14
LC50: .26
Method: OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year: 1997 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Test water was generated by adding the test substance in acetone to a larger volume of water which was stirred, allowed to settle, and then siphoned to a stock solution holding tank. This stock solution was then metered into exposure tanks for fish experiments. A preliminary study in trout was performed using nominal concentrations of the test substance of 0.1, 0.23, 0.51, 1.1, and 2.5 mg/L. Mortality rates were 100% at the highest level by Day-3, and was 80% by Day-7 at 1.1 mg/L.

In the definitive phase, duplicate test tanks contained 10 trout each, Test substance nominal concentrations of 0, 0.094, 0.19, 0.38, 0.75, and 1.5 mg/L (ppm) were chosen. Chemical analysis (HPLC) of the test substance in the test tanks on Days -0, -7 and -14 showed that mean concentrations for the 14-day test period were 0.062, 0.093, 0.14, 0.35, and 0.66 mg/L (ppm). Fish densities were 0.079 g biomass/L flowing test solution per day. Tank volume turnover for the flow-through system was 6.5/day. Fish were monitored daily for mortality and signs of erratic swimming behavior for 14-days during exposure. Body weights and lengths were recorded for representative fish prior to study initiation, and on all test fish on Day-14. LC50 values were calculated for 96-hours and 14-days.

Result: Fish died only at 0.35 and 0.66 mg/L concentrations; 0/20 and 1/20 died by Day-2 and 1/20 and 19/20 by Day -4 , respectively. Further, 100 % of the high dose (0.66 mg/L) fish died by Day-5 and 17/20 of the 0.37 mg/L fish by Day-14. Other findings at the two highest levels included darkened pigmentation of the fish, lethargic swimming behavior, and loss of equilibrium. There were test substance-related effects on 14-day body lengths and weights in the 0.35 mg/L group. The calculated LC50 for the test substance in the study at 96-hours was 0.48 mg/L and 0.26 mg/L at 14-days. The No Observed Effect Concentration (NOEC) was 0.14 mg/L at 96-hours and 14-days.

Reliability: (1) valid without restriction
31-JUL-2000

(38)

4.2 Acute Toxicity to Aquatic Invertebrates

Species:	Daphnia magna (Crustacea)
Exposure period:	48 hour(s)
Unit:	mg/l Analytical monitoring: yes
NOEC:	.36
EC50:	1.8
Method:	OECD Guide-line 202, part 1 "Daphnia sp., Acute Immobilisation Test"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding study used ten (10) 24-hour old daphnids exposed to nominal levels of 0, 13,22,36,60, and 100 mg/L of the test substance. Immobilization (15%) of the daphnids occurred at the highest level (100 mg/L). Sublethal lethargy was observed at all but the lowest test concentration (13 mg/L). Brown matter, apparently the test substance since brown precipitate was observed in the media, was observed to adhere to both surviving and non-surviving daphnids.

In the definitive phase, duplicate aquaria containing 10 daphnids each and test substance nominal concentrations of 0, 1.3, 2.2, 3.6, 6.0 and 10 mg/L (ppm) were prepared. Mean values for the test substance concentrations in the test media were determined by averaging chemical analyses (HLPC) of 0-hours and 48-hours.

Daphnia immobilization and aquaria observations were made at 24- and 48-hours following the study initiation. From these data, an Effective Concentration in one-half the organisims (EC50) and a No Observed Effect Concentration (NOEC) were estimated.

Result: Measured concentrations of the test substance ranged from 19 to 29% of nominal levels. At the highest concentration (1.8 mg/L), 25 % of the daphnids were immobilized at 48-hours of exposure. For the 0.68 and 1.1 mg/L groups, Five (5) % of the daphnids were immobile. No immobilization was observed at 0.20 and 0.36 mg/L exposures. Lethargic activity was not observed at any treatment level. Brown particulates, perhaps the test substance, were observed to adhere to the test daphnids, with some buoyed to the surface of the aquaria by this particulate material. The results indicated that the EC50 for the test substance was 1.8 mg/L. The No Observed Effect Concentration (NOEC) was shown to be 0.36 mg/L.

Reliability: (1) valid without restriction
31-JUL-2000 (28)

4.3 Toxicity to Aquatic Plants e.g. Algae

Species:	Selenastrum capricornutum (Algae)
Endpoint:	biomass
Exposure period:	72 hour(s)
Unit:	µg/l Analytical monitoring: yes
NOEC:	4.3
EC10:	4.3
EC50:	18
Method:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year:	1996 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding trial used nominal levels of 0, 1,10, 100, and 1000 ug/L (ppb) of the test substance and a solvent control in algae cultures (approximately 1x104 cells per flask). Following 72-hours incubation, algal cell densities were determined using a hemacytometer. Values were 127,76,109,69 and 1%, respectively, of the solvent control response. These values were used to set exposures for the definitive phase.

In the definitive phase, triplicate algal cultures were exposed to the test substance at nominal concentrations of 16, 31, 63,130, 250, and 500 ug/L (ppb). Cell densities were monitored at 24-, 48-, and 72-hours following study initiation. From these data, EC50 (50% decrease) values for Biomass (EbC50) and Growth Rate (ErC50) were calculated. Test substance concentrations in the test media were determined at 0- and 72-hours using HLPC. The mean concentrations were 7.5, 13, 14, 28, 50, and 79 ug/L (ppb).

Result: The inhibitions of algae Growth Rates for the test substance in the definitive 72-hour study were 0, 2, 15, 20, 32, and 38% (relative to pooled control values) for the measured test substance concentrations of 7.5, 13, 14, 28, 50, and 79 ug/L (ppb). Corresponding inhibitions of Biomass generation were 15, 41, 59, 63, 81, and 91%. Individual cell appearances were found microscopically to be normal for surviving cells except cellular bloating was noted at the highest exposure level. Calculations indicated that the ErC50 for the test substance was > 79 ug/L (ppb) while the EbC50 was 18 ug/L (ppb). The No Observed Effect Concentrations (NOECs) were assumed to be equivalent to EC10 values, and accordingly were EbC10 = 4.3 ug/L (ppb) and ErC10= 31 ug/L (ppb).

The EC50 values for the test substance ranged from 18 to > 79 ug/l (ppb) for Biomass increases and Growth Rates. The NOECs ranged from 4.3 to 31 ug/L (ppb) for these parameters.

Reliability: (1) valid without restriction
31-JUL-2000 (31)

Species: Selenastrum capricornutum (Algae)
Endpoint: growth rate
Exposure period: 72 hour(s)
Unit: µg/l Analytical monitoring: yes
NOEC: 31
EC10: 31
EC50: > 79
Method: OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year: 1996 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: A range-finding trial used nominal levels of 0, 1,10, 100, and 1000 ug/L (ppb) of the test substance and a solvent control in algae cultures (approximately 1x104 cells per flask). Following 72-hours incubation, algal cell densities were determined using a hemacytometer. Values were 127,76,109,69 and 1%, respectively, of the solvent control response. These values were used to set exposures for the definitive phase.

In the definitive phase, triplicate algal cultures were exposed to the test substance at nominal concentrations of 16, 31, 63,130, 250, and 500 ug/L (ppb). Cell densities were monitored at 24-, 48-, and 72-hours following study initiation. From these data, EC50 (50% decrease) values for Biomass (EbC50) and Growth Rate (ErC50) were calculated. Test substance concentrations in the test media were determined at 0- and 72-hours using HLPC. The mean concentrations were 7.5, 13, 14, 28, 50, and 79 ug/L (ppb).

Result: The inhibitions of algae Growth Rates for the test substance in the definitive 72-hour study were 0, 2, 15, 20, 32, and 38% (relative to pooled control values) for the measured test substance concentrations of 7.5, 13, 14, 28, 50, and 79 ug/L (ppb). Corresponding inhibitions of Biomass generation were 15, 41, 59, 63, 81, and 91%. Individual cell appearances were found microscopically to be normal for surviving cells except cellular bloating was noted at the highest exposure level. Calculations indicated that the ErC50 for the test substance was > 79 ug/L (ppb) while the EbC50 was 18 ug/L (ppb). The No Observed Effect Concentrations (NOECs) were assumed to be equivalent to EC10 values, and accordingly were EbC10 = 4.3 ug/L (ppb) and ErC10= 31 ug/L (ppb).

The EC50 values for the test substance ranged from 18 to > 79 ug/l (ppb) for Biomass increases and Growth Rates. The NOECs ranged from 4.3 to 31 ug/L (ppb) for these parameters.

Reliability: (1) valid without restriction
31-JUL-2000 (31)

4.4 Toxicity to Microorganisms e.g. Bacteria

Type: aquatic
Species: activated sludge
Exposure period: 30 minute(s)
Unit: mg/l Analytical monitoring: no

EC50: > 10000
Method: ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
Year: 1993 **GLP:** yes
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction

(6)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

Type: LD50
Species: rat
Strain:
Sex: no data
Number of Animals:
Vehicle:
Value: > 2000 mg/kg bw
Method: other: Directive 84/49/EEC, B.1
Year: 1990 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Reliability: (1) valid without restriction
01-AUG-2000 (7)

Type: LD50
Species: rat
Strain:
Sex: male/female
Number of Animals: 10
Vehicle: other: corn oil
Value: > 5000 mg/kg bw
Method: other: US EPA 40CFR798.2650, Oral Toxicity-Limit Test
Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Method: Five (5) male and five (5) female young adult rats (Sprague-Dawley) were administered a single dose of the test substance by gavage. The test substance was dispersed in corn oil (Sigma Chemical Company) and administered at a dosage of 5000 mg/kg. The animals were observed for clinical signs of toxicity at approximately 1-, 4- and 24-hours following administrations on the day of dosing and daily thereafter for 14-days. Body weights were recorded on Day-0, Day-7 and Day-14. All animals were subjected to a gross necropsy at study termination.

Result: One (1) animal died during the 14-day observation period. Clinical signs observed included decreased activity, decreased muscle tone, and diarrhea. No significant impairment on body weight gains were noted in either the male or female rats. Necropsy of the animal that died during the study revealed discolored kidneys, spleen, and liver. No visible lesions were observed in any of the animals at terminal necropsy. The estimated acute oral LD50 (combined sexes) for the test substance was determined to be > 5000 mg/kg.
Reliability: (1) valid without restriction
01-AUG-2000 (20)

Type: LD50
Species: rat
Strain:
Sex:
Number of
Animals:
Vehicle:
Value: > 4000 mg/kg bw
Method: other
Year: 1959 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Result: No animals died at the single high dose of 4000 mg/kg.

Reliability: (4) not assignable
01-AUG-2000 (39)

5.1.2 Acute Inhalation Toxicity

5.1.3 Acute Dermal Toxicity

Type: LD50
Species: rabbit
Strain:
Sex: male/female
Number of
Animals: 10
Vehicle: other
Value: > 2000 mg/kg bw
Method: OECD Guide-line 402 "Acute dermal Toxicity"
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Albino rabbits (five males and five females) were shaved in the caudal portion of the animals' trunks. One (1) day later, a 2000 mg/kg dose of 40 mesh test substance (obtained by grinding in mortar/pestle) was placed onto the skin sites (approximately 10% of the body surface areas). The application sites were then covered with gauze, plastic, and elastic wraps and finally secured with non-irritating tape. After 24-hours of skin contact to the exposure areas, the gauze patches were removed and adhering test substance removed with moistened gauze. Skin test sites were scored for signs of erythema (redness) and edema (swelling) according to Draize procedures from Day-1 to Day-14 following cessation of exposures. Animals were observed for adverse clinical signs, mortality, and body weights (Day-0, Day-7, and Day-14). Necropsies were performed on the final day of observations (Day-14).

Remark: A limit test

Result: The test substance induced no deaths or apparent adverse clinical signs. Mild irritation (Grades 1,2 erythema; Grade 1 edema) was seen at skin sites of treated rabbits for periods ranging from Day-1 to Day-10. Staining of skin was noted due to the dark color of the test substance. A body weight decrease was seen in one (1) of the ten (10) rabbits between Day-7 and Day-14. No compound-related non-dermal findings were observed in the study. No mortality or adverse clinical/necropsy changes were observed associated with the test substance. The dermal LD50 for the test substance was shown to be > 2000 mg/kg.

Reliability: (1) valid without restriction
01-AUG-2000 (27)

5.2.1 Skin Irritation

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classification: not irritating
Method: OECD Guide-line 404 "Acute Dermal Irritation/Corrosion"
Year: 1991 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Remark: Exposure period: 4 hours
Reliability: (2) valid with restrictions
(8)

Species: rabbit
Concentration:

Exposure:
Exposure Time:
Number of
Animals:
PDII:
Result: not irritating
EC classification: not irritating
Method: other: A 20% suspension of the material was applied to the shaved test site of six albino rabbits.
Year: 1959 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)
Reliability: (4) not assignable
(39)

Species: rabbit
Concentration: undiluted

Exposure: Occlusive
Exposure Time: 4 hour(s)

Number of Animals: 6
PDII: .46
Result: slightly irritating
EC classification: not irritating
Method: Draize Test
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Albino rabbits (six females) were shaved in the caudal Portion of the animals' trunks. One (1) day later, 0.5 grams of 40 mesh test substance (obtained by grinding in mortar/pestle) was placed on a one (1) inch squares of cotton gauze, moistened with water, applied to the skin sites, and secured with non-irritating tape. After 4-hours of skin contact exposures, the gauze patches were removed and adhering test substance removed with moistened gauze. Skin test sites were scored for signs of erythema (redness) and edema (swelling) according to Draize procedures at 1-, 24-, 48-, and 72-hours following cessation of exposures. Gross necropsies were performed on the animals following final scoring of the skin sites.

Result: The test substance induced no deaths or apparent adverse clinical or postmortem signs. Slight erythema (redness) was seen at skin sites of five (5) out of six (6) treated rabbits for maximum periods ranging from 1- to 48-hours. Staining of skin was noted due to the dark color of the test substance. The calculated irritation score was 0.46. The test results indicate an irritation rating as a "SLIGHT IRRITANT" and as a "NON-CORROSIVE".

Reliability: (1) valid without restriction

(26)

5.2.2 Eye Irritation

Species: rabbit
Concentration:
Dose:
Exposure Time:
Comment:
Number of Animals:
Result: not irritating
EC classification: not irritating
Method: OECD Guide-line 405 "Acute Eye Irritation/Corrosion"
Year: 1991 GLP: yes
Test substance: as prescribed by 1.1 - 1.4
Remark: Exposure period: 24 hours

Reliability: 2) valid with restrictions
(8)

Species: rabbit
Concentration: undiluted
Dose: .1 ml
Exposure Time: 72 hour(s)
Comment: rinsed after (see exposure time)
Number of Animals: 9
Result: slightly irritating
EC classification: irritating
Method: Draize Test
Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: The eyes of albino rabbits (9-both genders) were examined using fluorescein dye and UV light for evidence of corneal damage and dye retention. Animals found to be acceptable received approximately 0.06 grams (0.1 mL) of 40 mesh test substance (obtained by grinding in mortar/pestle) applications to the right eyes. After 30-seconds of eye contact to the test substance, a water rinse was applied to three (3) of the nine (9) rabbits in an attempt to minimize chemical irritation. Left eyes were untreated and served as control sites. Eyes were assessed for signs of gross corneal, iridal, or conjunctival injury according to Draize procedures at 1-, 24-, 48-, and 72-hours (7-days for one (1) rabbit with eye damage at 72-hours). Fluorescein dye exams were conducted at 24-hours.

Result: The test substance induced no adverse clinical signs. No corneal damage was induced in any of the unrinsed rabbits although one (1) out of six (6) rabbits exhibited dye retention judged to be non-chemically related. Conjunctival {six (6) of six (6) and iridal (one (1) of six (6)}changes were seen in unrinsed rabbits primarily at the 1-hour inspection. All adverse findings were resolved by 72-hours except for one (1)rabbit with conjunctival redness which resolved by 7-days.The rinsed group exhibited some conjunctival irritation up to 72-hours. Irritation mean scores for unrinsed rabbits ranged from 8.2 (1-hour) to 0.33 (72-hours) to 0.0 (7-Days). Rinsed rabbits scores were 5.3 (1-hour) to 0.0 (72-hours). The test substance produced a mild irritation in rabbit eyes which was shown to be reversible. The test substance is considered to be a "MILD IRRITANT" to the eye.

Reliability: (1) valid without restriction

(25)

5.3 Sensitization

Type:	Guinea pig maximization test			
Species:	guinea pig			
Concentration:	Induction	5	% active substance	intracutaneous
	Induction	100	% active substance	intracutaneous
	Challenge	25	% active substance	occlusive epicutaneous
Number of Animals:	36			
Vehicle:				
Result:	sensitizing			
Classification:	sensitizing			
Method:	OECD Guide-line 406 "Skin Sensitization"			
Year:	1995		GLP: yes	
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)			

Method: Two (2) range finding trials (topical and intradermal injection) in two (2) male and two (2) female shaved albino guinea pigs were run which showed that the test substance at concentrations of 100% and 5% were appropriate for the definitive study, respectively. In the induction phase of the test, twenty test animals were given pairs of intradermal (0.1 mL) injections of 1) Freund's adjuvant, 2) %5 test substance in 0.5% acetone in propylene glycol, and 3) test substance + Freund's adjuvant at opposite sites from the animals' dorsal midline on Day-0. Appropriate negative and positive {2,4-Dinitro-1-chlorobenzene(DNCB)}controls were run on other animals. Topical induction exposures (48-hours) with site occlusion were done 7-days later following 24-hours test site exposure to Sodium lauryl sulfate.

Challenge (dermal)exposures were performed on Day-21 with both 25% (in acetone/mineral oil) and 100% test substance for 24-hours. Test animals were graded for dermal signs on the first and 2nd days following the challenge dosing. A dermal rechallenge trial was conducted on Day-28 by applying the test substance(25 and 100%) to these same animals. Dermal examinations were again performed one (1) and two (2) days later.

Result: The test substance induced no adverse clinical signs. Weak skin responses (erythema and edema) were observed in 25% test substance-treated challenge controls and in test substance-induced animals. Mean scores were not significantly different from the controls although a greater number of induced animals exhibited "slight but confluent or moderate patchy erythema". The test substance at 100% produced the same results. However, upon rechallenge of these animals 7-days later with 25 and 100% test substance, severities of dermal responses increased in test substance induced animals as did the mean dermal scores (0.8-1.0) relative to challenge (non-induced) controls (0.0-0.3). The positive control agent (DNCB) produced dermal scores at 24- and 48-hours of 0.3 and 0.5 for previously untreated animals versus scores of 2,5 for DNCB-induced guinea pigs. The test substance is considered to be a contact sensitizer.

Reliability: (1) valid without restriction

(24)

5.4 Repeated Dose Toxicity

Species:	rat	Sex: male/female
Strain:	Fischer 344	
Route of admin.:	oral feed	
Exposure period:	28 days	
Frequency of treatment:	Daily	
Post. obs. period:	2 weeks	
Doses:	0, 7.5, 30 and 120 mg/kg/day	
Control Group:	yes, concurrent vehicle	
NOAEL:	7.5 mg/kg	
LOAEL:	30 mg/kg	
Method:	other: Oral 4-week dietary study	
Year:	1996	GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)	

Method: The test substance was prepared by grinding in a coffee mill, sieved through a 125 um mesh screen and mixed with rodent diet NIH-07 at 0, 120, 470, 1900 ppm (0, 7.5, 30, and 120 mg/kg/day). Stability, homogeneity, and dose verification were performed to confirm compliance with protocol. The prepared dosed feed was presented to 14 male and 14 female rats (Fischer 344) per test group at twelve weeks of age for four (4) weeks. Six (6) rats/sex/group were held for post-exposure in two (2) week recovery groups. Test rats were monitored for body weights, feed consumption, and clinical signs. Collections were performed on six (6) or three (3) rats/sex/group at 28-days and 42-days sacrifice periods for blood (hematologies and clinical chemistries) and urinalyses, respectively. Necropsies were performed on all rats, and organs were weighed (liver, kidneys, pituitary, uteri, heart, brain, spleen, thyroids, adrenals, testes, and ovaries). These and other major organs were preserved in formalin, stained with H&E, and subjected to microscopic evaluations. Liver, kidney, and urinary bladder slices were subjected to immunohistochemical staining for proliferating cell nuclear antigen (PCNA) for assessment of cellular division.

Result: The test substance was shown to be completely stable in diets for 46-days. Mixing procedures produced homogeneous diets that were found within 10% of target concentrations. No compound-related deaths occurred. The body weights were not affected in male rats whereas the high dose female rats displayed 5% body weight decreases during study weeks two (2) through four (4). Food consumption was decreased in the high dose males and in the mid- and high dose females mainly during study weeks two (2) through four (4).

Various test substance-induced hematological changes occurred that included: increased mean corpuscular volumes and decreased mean corpuscular hemoglobin concentrations (high dose males and females) and blood bilirubin and cholesterol increases (high dose males and females). Most blood endpoints tended to approach control levels during week two (2) of the recovery period. No dose-related urinary changes were seen. Organ weight increases were seen at 28-days for liver and kidneys(high dose males and females; mid-dose females) and heart and spleen (high dose females). Only the kidney weights did not reach control levels by 42-days. There were no gross tissue or microscopic changes related to the test substance. Proliferating cell nuclear antigen (PCNA) exams showed cell division changes for: increases for liver cells (High dose males and females and mid-dose males at 28-days only); changes for kidney cells (decreases in high dose females at 28-days and increases in high dose males and females at 42-days; and increasing trend in urothelial cells in bladder (low and mid-dose males and females at 28-days). Macrocytic anemia was the primary change in rats related to the test substance administration. This change was reversible within 2 weeks following dietary exposure as were liver weight and serum cholesterol elevations. These changes were very minor, and had no apparent toxicological significance in this study. The lack of dose-responsiveness in the PCNA data provides results of uncertain importance to the assessment of the toxicity of this test substance.

Reliability: (1) valid without restriction
02-AUG-2000

(11)

Species:	rat Sex: male/female
Strain:	other: Fischer 344/N TacfBR
Route of admin.:	gavage
Exposure period:	21 days
Frequency of treatment:	Daily
Post. obs. period:	
Doses:	0, 0.1, 0.3, 1.0, and 3.0 g/kg/bw
Control Group:	yes, concurrent vehicle
LOAEL:	100 mg/kg bw
Method:	other: Oral 3-Week Range-Finding Study
Year:	1994 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)

Remark: A 4-week diet-study was also conducted.

Result: Doses of 1.0 and 3.0 g/kg/day of WINGSTAY 100 (mixed diaryl-p-phenylenediamines) were administered by gavage for up to 6 days were lethal for male and female F344 rats. The only pertinent gross finding of all unscheduled deaths was the paleness of most external surfaces and viscera. The mid-low (0.3 g/kg/day) and low(0.1 g/kg/day) doses caused time and dose related significant body weight loss, liver weight increase and hepatocellular labeling index increase at 0.1 g/kg. Therefore, in the subchronic studies, the recommended daily dose of WINGSTAY 100 (mixed diaryl-p-phenylenediamines) should not exceed 100 mg/kg/day, if administered by gavage.

Test substance Preparation: The test substance was prepared in an olive oil suspension for dosing.

Reliability: (1) valid without restriction
02-AUG-2000

(5)

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test

System of testing: Ames/E. coli preincubation; Salmonella typhimurium TA-98, 100, 1535, 1537, 1538, and WP2 uvrA

Concentration: Salmonella stains without S9 activation: 0.167, 0.5, 1.67, 5, 16.7, and 50 ug/plate; Salmonella strains with S9 activation: 1.67, 5, 16.7, 50, 167, and 500 ug/plate; E.coli with/without S9 activation: 1.67, 5, 16.7, 50, 167, and 500 ug/plate

Metabolic activation: With and without

Method: other: Japan's Industrial Safety & Health Law, a combination of OECD Guidelines 471 and 472.

Result: Positive. The test substance was shown to cause mutations in Ames/Salmonella strains TA1538 and TA98 with S9 activation.

In a preliminary assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, TA98, TA100, and WP2 uvrA with S9 metabolic activation, and in tester strains TA1535, TA1538, TA98, TA100 and WP2 uvrA without S9 activation, approximated the concurrent negative controls. However, statistically significant, increases in reverent frequencies, to approximately 1.7- to 2.5-fold control values, were observed in tester strain TA1538 with S9 metabolic activation and in tester strain TA1537 without S9 metabolic activation. In addition, the increases observed in strain TA1538 with S9 metabolic activation were dose dependent.

In a confirmatory assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA100, and WP2 uvrA with metabolic activation, and in tester strains TA1535, TA1538, TA98, TA100, and WP2 uvrA without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with metabolic activation. Statistically significant increases in reverent frequencies, to control values, also were observed in tester strain TA98 without S9 metabolic activation. However, these latter increases apparently were not dose related.

The test substance was re-evaluated in all five Salmonella strains with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation. Reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, and TA100 with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation, approximated or were less than control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1538 and TA98 with S9 metabolic activation. All positive and negative control values in all assays were within acceptable limits.

Year: 1993 GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(16)

Type: Ames test

System of testing: Ames/Salmonella-E.coli Liquid Pre-incubation Assay in Salmonella strains TA1535, TA1537, TA1538, TA98, and TA100 And in E.coli strain WP2 uvrA.

Concentration: Salmonella strains with S9: 1.67, 5, 16.7, 50, 167, and 500 ug/plate; Salmonella strains without S9: 0.167, 0.5, 1.67, 5, 16.7, and 50 ug/plate; E.coli with/without S9: 1.67, 5, 16.7, 50, 167, and 500 ug/ plate.

Metabolic activation: With and without

Method: other: Japan's Industrial Safety & Health Law, a combination of OECD Guidelines 471 and 472.

Result: Positive. The test substance was shown to cause mutations in Ames/Salmonella strains TA1537, TA1538 and TA98 with S9 metabolic activation.

In a preliminary assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA1537, TA100, and WP2 uvrA with and without S9 metabolic activation approximated the concurrent negative controls. However, statistically significant, increases in reverent frequencies, to control values, were observed in tester strains TA1538 and TA98 with S9 metabolic activation. In addition, the increases observed in strain TA1538 with S9 metabolic activation were dose dependent.

In a confirmatory assay, reverent frequencies for all doses of the test substance in tester strains TA1535, TA100, and WP2 uvrA with metabolic activation, and in tester strains TA1535, TA1537, TA1538, TA100, and WP2 uvrA without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with metabolic activation. Statistically significant increases in reverent frequencies, to control values, also were observed in tester strain TA98 without S9 metabolic activation. However, these latter increases apparently were not dose related.

The test substance was re-evaluated in all five Salmonella strains with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation. Reverent frequencies for all doses of the test substance in tester strains TA1535, and TA100 with S9 metabolic activation, and in tester strain TA98 without S9 metabolic activation, approximated control values. Statistically significant, dose-dependent increases in reverent frequencies, to control values, were observed in tester strains TA1537, TA1538, and TA98 with S9 metabolic activation. All positive and negative control values in all assays were within acceptable limits.

Year: 1994 GLP: yes

Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(17)

Type:	Cytogenetic assay
System of testing:	Chromosomal aberration assay in CHO cells
Concentration:	0.4, 2, 4, and 25 ug/mL
Metabolic activation:	With and without
Result:	Negative. The test substance was judged negative (non-clastogenic)based on its inability to reproducibly induce dose-related increases in structural chromosomal aberrations in CHO cells. Analysis of the data for the 24-hour treatment with the test substance indicated that there were statistically significant dose-related increases in the frequency of aberrations/cell and proportion of aberrant metaphases at doses 2 and 4 ug/mL. The data for the 2 and 4 ug/mL doses produced a statistically significant linear trend when analyzed by the Cochran/Armitage Linear Trend Test. To verify the biological significance of this finding, the 24-hour treatment was repeated. In the confirmatory assay, the test substance was re-evaluated at doses of 25 ug/mL with S9 metabolic activation (5-hour treatment) and 0.4, 2, and 4 ug/mL without S9 metabolic activation (24-hour treatment). Analysis of the data for the 5-hour treatment did not produce statistically significant increases in aberrations/cell or in proportion of aberrant metaphases. Analysis of the data for the 24-hour treatment indicated a statistically significant increase in aberrations/metaphase at the mid-dose (2 ug/mL) with S9 metabolic activation but there were no significant increases in the proportion of aberrant metaphases. However, when the data for 2 ug/mL (0.045 + or - 0.208) were compared to the untreated control data (0.025 + or - 0.157) or to Pharmakon historical acetone data (0.034 + or - 0.021), there were no statistically significant increases in the frequency of aberrations/metaphase. Therefore, the positive finding in the t-test for 2 ug/mL was considered a statistical artifact with no biological significance. There were no other statistically significant increases in aberration/metaphase or in the proportion of aberrant metaphases at any of the remaining dose levels for the 24-hour treatment.
Method:	OECD Guide-line 473 "Genetic Toxicology: In vitro Mammalian Cytogenetic Test"

In the structural Chromosomal Aberration assay, duplicate cultures were established for each dose level. Three treatment schedules were used: a) First set of cultures were treated for 5-hours with the appropriate dose of the test sample in Ham's F12 serum free (F12SF) medium either in the presence or absence of S9 metabolic activation along with concurrent negative and positive controls followed by three (3) Puck's saline washes and medium replacement; b) Second set of cultures were treated for 24-hours with the test substance or control articles in Ham's F12 medium containing five (5) % serum (F12FCM5%) without S9 metabolic activation, and; c) Third set of cultures were treated for 48-hours with the test substance or control articles in F12FCM5% medium without S9 metabolic activation. Two (2) to three (3) hours prior to harvest, Colcemid (2X10-7M) was added to all sets of cell cultures to arrest dividing cells in metaphase. CHO cells were harvested at the appropriate time and metaphase slides were prepared and stained.

The data from one hundred metaphases from each culture (200 metaphases per dose point) were pooled for statistical analysis. Data were evaluated by using the chi-square of aberrant versus normal cells while comparing each dose level to its concurrent negative control. The data were also analyzed for statistical significance by pairwise t-tests comparing the number of aberrations per cell in each treated dose versus the negative control.

Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
20-FEB-2001

(19)

Type: DNA damage and repair assay
System of testing: E. coli Pol A1- Liquid Suspension Assay

Concentration:

Metabolic activation: Without

Result: Positive
Method: Other
Year: 1980 GLP: no

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(33)

Type: other: Transformation Assay
System of testing: Balb/3T3 In Vitro Transformation Assay

Concentration: .01 ug/ml to 1.0 ug/ml

Metabolic activation: Without

Result: Negative
Method: other
Year: 1981 GLP: no

Test substance: Nailax (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although this study was probably not conducted to GLP, the test parameters used were based on a known and well established procedure.
04-AUG-2000

(12)

Type: other: Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes
System of testing: Hepatocytes form male Fischer 344 (F344/Crl) rats

Concentration: Slightly above their limits of solubility

Metabolic activation: Without

Result: Negative. In all the Unscheduled DNA Synthesis Assay (UDS) trials, the three (3) negative controls {the untreated cells control, F, and Dimethylsulfoxide (DMSO)} had negative values for Net Nuclear Gain (NNG) counts (<0). A positive control, 2-Aminofluorene (2-AF) was positive for induction of UDS; the mean NNG counts were 45.92 and 58.99 in the first and second assays, respectively, indicating assay validity. (i.e., hepatocytes were capable of metabolic activation and DNA repair). The positive control responses occurred at

toxic levels. UDS assay results for NNGs were in the range of -26 to -46, demonstrating a lack of UDS activity for the three (3) condensation products at concentrations greater than their solubilities in the test media. The results indicated that, under controlled laboratory conditions, the condensation products from the reaction of 1.4-Benzenediamine, N,N', mixed Ph and tolyl. derivs. with Dicyclopentadiene were negative for induction of UDS in rat hepatocytes at concentrations up to and greater than their solubilities. This assay demonstrated a lack of genetic activity in this mammalian DNA-repair test system.

Method: other: Unscheduled DNA Synthesis Assays (UDS) with Rat Hepatocytes on Test substance Condensation Products. The test substance, 1,4-Benzenediamine, N.N'-mixed Ph and tolyl. derivs., was reacted with Dicyclopentadiene in varying ratios, resulting in three condensation products. Each of these condensation products were subjected to independent in vitro unscheduled DNA synthesis (UDS) assays with hepatocytes from male Fischer 344 (F344/Crl) rats. All three (3) condensation products were tested at concentrations slightly above their limits of solubility in the tissue culture medium. Hepatocytes were exposed to test substances for 18-20 hours to allow bioactivation and DNA repair. The assay was based on the incorporation of 3H-thymidine into the hepatocyte's DNA during repair of DNA-damage. This incorporation was monitored by counting Net Nuclear Grains (NNG) formed on photographic emulsion placed on the cells adhering to glass slides. Criteria for a positive response included : (a) Significant increase in number of grains at two (2) levels of exposure above negative control levels, (b) A dose-responsiveness in grain counts up to toxic levels of exposure, and (c) At least one (1) value for NNG that is five (5) or above. A negative response is reported for NNG's that are <0, and an equivocal or inconclusive response are results that are 0<#<5.

Year: 1999 GLP: yes

Test substance: The test substance, 1,4-Benzenediamine, N.N'-mixed Ph and tolyl. Derivs. condensation products with Dicylopentadiene

Reliability: (1) valid without restriction
07-AUG-2000

(37)

5.6 Genetic Toxicity 'in Vivo'

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex:
Strain:
Route of admin.: Oral feed
Exposure period: 24 hours
Doses: 50 ug/ml and 10 ug/ml
Result: Negative. Negative under conditions of the assay
Method: other: Drosophila melanogaster (Fruit Fly) System
Year: 1979 GLP: no

Test substance: Nailax B (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(32)

Type: Drosophila SLRL test
Species: Drosophila melanogaster Sex:
Strain:
Route of admin.: Oral feed
Exposure period: 24 hours
Doses: 0.05 mg/ml and 0.63 mg/ml
Result: Negative. Negative under conditions of the assay
Method: other: Drosophilia SLRL Assay
Year: 1979 GLP: no

Test substance: Nailax (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although the study was old and was not conducted to GLP, the test parameters were based on a scientifically sound procedure for that time period and the study was properly conducted.
04-AUG-2000

(13)

Type: Micronucleus assay
Species: Mouse Sex: male/female
Strain: CD-1
Route of admin.: i.p.
Exposure period: single dosing
Doses: 0, 250, 1250, 2500 mg/kg test chemical; 0.5 g/kg TEM (+ control)
Result: Negative. There were no statistically significant depressions in the PCE/NCE ratios in any groups of mice except for the 2500 mg/kg group at 48-hours sacrifice time ($p<0.01$) which was an indication that the test substance had reached the bone marrow and was toxic to erythrocytes.

Analysis of the micronucleus data for the groups treated with the test substance indicated that there were no statistically significant increases in the frequency of micronucleated PCEs. The test substance was judged negative (non-clastogenic) based on its inability to induce micronucleated PCEs.

Method: OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"

Nine (9) groups of mice (CD-1) were acclimated to laboratory conditions for 25-days prior to initiation of the study. The mice were randomized by body weight and assigned to groups using a computer-generated random number list.

Each group of mice was comprised of ten (10) animals(five (5) males/five (5) females). Each mouse received a single interperitoneal dose at 10 mL/kg of body weight. The test substance at dose levels of 250, 1250, and 2500 mg/kg was administered to three (3) groups of mice which were sacrificed at 24-, 48-, and 72-hours post dose. Concurrently, the negative control, Dimethylsulfoxide (DMSO)/corn oil, was administered, as dose volume of 10 mL/kg of body weight, to three (3) groups of mice. A group of these mice were included in each sampling time. The positive control, Triethylenemelamine at 0.5 mg/kg, was administered to one (1) group of mice and sacrificed at 24-hours post dose.

All mice were sacrificed and their femurs were removed. Their bone marrow was removed by flushing. Smears were made of the suspended cells.

One (1) thousand young erythrocytes were evaluated for a change of ratio of polychromatic erythrocytes (PCE) to normochromatic cells (NCE).

Year: 1993 GLP: yes
Test substance: as prescribed by 1.1 - 1.4

Reliability: (1) valid without restriction
04-AUG-2000

(18)

Type:	Other: 32P Postlabeling Assay for Detection of Adduct Formation in Rat DNA
Species:	rat Sex: male/female
Strain:	other: Fischer 344/N TacfBR
Route of admin.:	Gavage
Exposure period:	7 days
Doses:	0., 0.3, 1.0, and 3.0 g/kg/bw
Result:	Negative. Under conditions of the study, the test substance did not induce DNA-adducts in the liver and urinary bladder DNA of rats.
Method:	Other: 32P Post-Labeling Assay for DNA Adduct Formation
Year:	1995 GLP: yes
Test substance:	Wingstay 100 (mixed diaryl-p-phenylenediamines)
Remark:	The purpose of the study was to determine the potential of WINGSTAY 100 (mixed diaryl-p-phenylenediamines)to bind covalently to liver and urinary bladder DNA of male and female rats after in vivo administration of WINGSTAY 100.
Result:	Under conditions of the study, the test substance did not induce DNA-adducts in the liver and urinary bladder DNA of rats.
Reliability:	(1) valid without restriction
07-AUG-2000	

(4)

5.7 Carcinogenicity

Species:	rat	Sex: male
Strain:	Fischer 344	
Route of admin.:	oral feed	
Exposure period:	38 weeks	
Frequency of treatment:	Daily	
Post. obs. period:		
Doses:	1900 ppm	
Result:	Negative. The test substance exerted toxicity to the erythropoietic system, but there was an absence of tumor initiating or promoting activity.	

Control Group: yes, concurrent vehicle
Method: other: Accelerated bioassay (ABA)

The accelerated bioassay (ABA) was conducted on male F344 rats for 38 weeks. The target sites chosen for the ABA were liver and urinary bladder and the dose of the test substance was 1900 ppm as previously established to be a toxic dose. The liver tumor initiator was Diethylnitrosamine (DEN) and the urinary bladder initiator was N-Butyl N(4-hydroxybutyl)nitrosamine(BBN). The initiators, which included the test substance as a possible initiator, were administered during the first 14-weeks followed by the promoters. The promoters, Phenolbarbital (PB) for the liver and Nitrilotriacetate (NTA)for the urinary bladder and the test substance as a possible promoter, were administered during last 24-weeks after the test substance. The study had 11 test groups, including a negative control. The critical comparisons for initiation activity were conducted between Group Three (3) (PB) and Group Six (6) (Test substance + PB) for the liver and Group Eight(8) (NTA) and Eleven (11) (Test substance + NTA) for the urinary bladder. The critical comparisons for promoting activities were conducted between Group Two (2)(DEN) and Group Five (5) (DEN + Test substance) for the liver and Group Seven(7) (BBN) and Group Ten (10) (BBN + Test substance) for the urinary bladder. There were 26- and 38-week sacrifices.

Once daily, clinical observations were made and on scheduled body weighing days, a thorough palpation was performed on all animals. Body weights were recorded weekly from the first week of dosing until scheduled sacrifice at 26-weeks, and every 2-weeks thereafter.

At the two (2) scheduled sacrifices, all animals were subjected to a complete gross postmortem examination, The liver and kidneys were weighed. Liver, urinary bladder, kidneys and any grossly observed change or lesions were sampled, fixed, processed, cut and stained for microscopic examination. Tissue samples were taken from each of the three (3) liver lobes. NBF was used to inflate the urinary bladder at necropsy. All animals found dead or those killed in extremis were submitted to a complete gross postmortem examination. No organ weights were taken. The mean number of neoplasms per animal, the biggest diameter of carcinomas (in mm), the average diameter of carcinomas (in mm), and the degree of severity of carcinomas were recorded.

In order to assess proliferation, separate liver and urinary bladder sections were fixed in NBF, were cut and stained for PCNA. Subsequently, they all were aquatinted according to the method described above.

Statistical analyses were performed on weekly body weights, final body weights, absolute and relative liver and kidney weights, tumor incidence and PCNA data using methods described above.

Year: 1996 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction

(2)

Species: rat Sex: male/female
Strain: Fischer 344
Route of admin.: oral feed
Exposure period: 52 weeks
Frequency of treatment: Daily
Post. obs. period: 12 weeks
Doses: 53, 310, 1900 ppm
Result: Negative. No test substance related deaths occurred, although the high dose of 1900 ppm caused a decrease in body weight gain and food consumption in both genders. Red blood cell mean corpuscular volume was significantly increased at 38-weeks, accompanied by a significant decrease in mean corpuscular hemoglobin concentration.

At 52-weeks, the red blood cell count and hemoglobin values were also significantly decreased in high dose animals of both genders. Total bilirubin and cholesterol were increased in high dose animals at 38- and 52-week sacrifices. During the 3-month recovery, hematology parameters, bilirubin and cholesterol returned to control values. Total protein was reduced in high dose animals of both genders, throughout the entire exposure and recovery periods. The test substance also produced increases in relative liver, spleen, heart, and kidney weights in high dose animals. Both genders of all test substance groups exhibited significant increases in urothelial cell proliferation (measured by PCNA)and adaptive hyperplasia. No regenerative hyperplasia, prenoplasia, or neoplasia were present. There were microscopic evidence of extramedullary erythropoiesis in the spleen and liver of high dose animals in both genders; otherwise, no other pertinent microscopic findings were evident. The test substance exerted toxicity to the erythropoietic system, but displayed no carcinogenic activity.

Control Group: yes, concurrent vehicle

Method: other: One year study in male and female F 344 rats

The study used both genders of Fischer 344 (F344/N Tacf Br MPF) rats. There was a 38-week interim sacrifice in addition to 52-week, and 12-week post-exposure (recovery) sacrifice periods. The high dose in the study (1900 ppm) was the maximum tolerated dose identified in subchronic studies, in which there was no observable gender difference.

Once daily, cage side clinical observations were made, and on days scheduled for body weighing, a thorough body palpation was performed. Body weights were recorded one (1) week prior to initiation of exposure, weekly for weeks 1-13, and once every two (2) weeks thereafter. Food consumption was measured for weeks 1-13, and once every two (2) weeks thereafter. Indirect ophthalmoscopy was performed on all animals prior to exposure and during week-52.

During the three (3) sacrifices (at 38-, 52-, and 64-weeks), Five (5) rats/group/gender were used for hematology, clinical chemistry, and urinalysis. At scheduled sacrifices, all animals were subjected to a complete postmortem examination. Key organs were weighed and the tissues fixed in neutral buffered formalin (NBF), processed, cut, and stained with H&E. Tissue samples were taken from each of the three (3) liverlobes. NBF was used to inflate the urinary bladder at necropsy. All animals found dead and those killed in extremis were submitted to a complete gross postmortem examination. For these, no organ weights were taken, but all grossly observed changes and all key tissues were examined microscopically.

To assess cell proliferation, separate liver, urinary bladder and kidney sections were fixed in NBF, cut, and stained for proliferating cell nuclear antigen (PCNA). The quantitation of PCNA-positive nuclei in the immuno-stained sections of these tissues, was performed from 38-, 52-, and 62-week sacrifices. Next, the proliferation index (PI) for the liver, urinary bladder, and kidney for each animal was calculated, representing the percentage of PCNA-positive nuclei out of the total number of hepatocellular, urothelial, or tubular nuclei counted. The results were subjected to appropriate statistical analysis.

Statistical analysis was performed on weeking body weights, food consumption data, absolute and relative organ weights, hematology, clinical chemistry, urinalysis, and PCNA data.

Year: 1996 GLP: yes

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction

(3)

5.8 Toxicity to Reproduction

Type: Two generation study
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure Period: F0 exposed during 10 weeks premating, 2 weeks of mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 males and females exposed for 10 weeks prior to mating.
Frequency of treatment: Daily
Premating Exposure Period:
male: 10 weeks
female: 10 weeks
Duration of test: 9 months
Doses: 0, 120, 400 or 1500 ppm.

Control Group: yes, concurrent no treatment

Method: OECD Guide-line 416 "Two-generation Reproduction Toxicity Study"

This study was designed in compliance with EPA GLP and USEPA FIFRA guidelines. Dose levels were established from a Range finding study at Research Triangle Institute which employed dietary levels of 120, 1900, and 5700 ppm of WINGSTAY 100 (mixed diaryl-p-phenylenediamines). The top level was lethal to dams and offspring, 1900 ppm induced one nonviable litter in 9 total, and thus, the top dose for the definitive study was decreased by 20% to assure high viability in test group. No effects were seen at 120 ppm.

This study used 30 Sprague Dawley rats/sex/dose (F0) exposed to diets containing 0, 120, 400 or 1500 ppm WINGSTAY 100 during 10 weeks premating, 2 weeks mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 litters were culled to 10 each at 4 days postnatal (PND) 30 other F1 males and females/group chosen for pairing, and fed WINGSTAY 100 as above for 10 weeks prior to mating. After mating/gestation of F1, the resulting F2 rats were delivered, and maintained through weaning period (to PND 21). Weekly body weights (BWs) and food consumption (FC), and daily clinical observations were recorded. Necropsies and histopathology (primary kidneys) were performed on selected rats from each sex/group/generation (all F0 and F1 dams at PND21, three F1 and F2 pups/test group at PND21). Remaining F1 and F2 rats were euthanized without examination. Data were collected on vaginal cytology, mating, pregnancy, litter, and pup parameters.

Remark: WINGSTAY 100 induced dystocia (difficult deliveries) in pregnant rats which may have led to prolonged gestation and increased perinatal deaths, decreased live births, and increased pup weights. In addition, polycystic lesions were observed at all dose levels. Prolonged gestation has previously been associated with the WINGSTAY component DPPD, and polycystic kidneys were observed in DPamine-treated rats. Based upon adult toxicities, reproductive and offspring endpoints, there was no NOEL for WINGSTAY 100 in this study.

Result: High dose females had decreased Body Weights (BWs) relative to other test groups throughout majority of study period. Mortality during gestation/lacation were: F0 dams- 0 in 24 pregnancies, 0/27, 3/24, 4/25; F1- 0/22, 0/23, 1/22, 1/24. Numbers of pregnancies with no live births: F0- 0, 1, 1, 10; F1- 0, 1, 1, 2. Gestational length: F0- 22.2 days, 22.4 days, 22.8*, 23.5*; F1- 22.2, 22.8*, 23.1*, 23.2* (* = statistically significant). The number of live pups/litter: F0-15.6, 14.1, 11.9, 7.6*; F1- 15.6, 13.7, 13.3, 10.8*. Pups weights (g) on PND 0: F0- 6.38, 6.79*, 6.93*, 6.63*; F1- 6.32, 6.89*, 6.99*, 6.63*.

WINGSTAY 100-related kidney lesions were observed grossly (as white or clear cysts) and microscopically (polycyctic findings with variable severity): F0 adults-males 0/0, 0/0, 0/0, 0/1 and females 0/0, 0/0, 0/2, 3/9; F1 weanlings-males 0/23, 1/25, 8/20, 10/11 and females 0/22, 5/26, 7/18, 11/11; F1 adults-males 0/30, 5/30, 10/30, 21/30 and females 0/30, 2/30, 1/30, 18/30; F2 weanlings-males 0/60, 3/64, 6/19, 15/16 and females 0/60, 5/64, 8/19, 15/15. The severity of kidney lesions were also dose related.

Year: 2000 GLP: yes

Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines) The test substance was prepared in an olive oil suspension and mixed with rodent diet NIH-07 for dosing.

Reliability: (1) valid without restriction
11-FEB-2001

(36)

Type: Two generation study
Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure Period: F0 exposed during 10 weeks premating, 2 weeks of mating, 3 weeks (gestation), and through the weaning (21 day) period. F1 males and females exposed for 10 weeks prior to mating.
Frequency of treatment: Daily
Premating Exposure Period:
male: 10 weeks
female: 10 weeks
Duration of test: 9 months
Doses: 0, 120, 400 or 1500 ppm.

Control Group: yes, concurrent no treatment

Method: Other: Derivation of Benchmark Dose from 2-Generation Rat Study
Test Substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Method: Bench Mark Responses (BMR) are estimations of doses inducing a discrete toxic response in a test population at an incidence within the range of 1-10%. The Bench Mark Dose (BMD) is represented as the 95% lower confidence limit (LCL) for a BMR, or as a Most Likely Estimate (MLE). In this project, data from the 2-generation reproduction study in rats on Wingstay 100 (RTI #65C-6429-400)(36) chosen for analyses were the (1) polycystic kidney lesions in F1 male adults and F1 female weanlings, and (2) gestational lengths (days) for F1 pregnant females.

Data for these endpoints at the 3 dose levels employed in the study were subjected to various analyses including Gamma, Multistage, Quantal Linear, Weibull, Probit, Logistic, and Quantal Quadratic (for quantal data – polycystic kidneys), and Power, Linear, and Polynomial models (continuous data – gestational lengths). Estimations were also made to derive "best fit" information for each model run. The methodology employed was according to the "Benchmark Dose Technical Guidance Document" (1996), EPA/600/P-96/002A.

Results: Most Likely Estimate (MLE) and 95% Lowest Confidence Limit (LCL) values were derived for the most sensitive toxic endpoints (observed graphically). The models that "best fit" polycystic data for F1 male adults and F1 female weanlings were the quantal linear and multistage procedures. The BMD 10% values (EPA default for quantal data) derived for F1 male adults are 7 mg/kg-day (LCL) and 9.3 (MLE), and for F1 female weanlings, the values are 3.7 and 6.0 mg/kg-day, respectively. The prolongation of parturition analysis for F1 females indicated that none of the models produced a good fit although there was good agreement amongst the 3 models tested, giving BMD 5% estimations of 160 (LEL) and 226 (MLE) mg/kg-day for this endpoint.

The Bench Mark Dose (10% incidence) developed for the the most sensitive endpoint (polycystic kidneys in F1 female weanling rats) in the 2-generation rat dietary study was 3.7 (95% Lower Confidence Limit) and 6.0 (Most Likely Estimate) mg/kg-day. These numbers are below the lowest exposure levels (and LOEL) found in the 2-generation study, and thus pose plausible estimates of a 10% incidence rate for this endpoint. These calculations provide a credible low dose benchmark that can be used as a basis for safety assessments in exposed populations.

(40)

5.9 Developmental Toxicity/Teratogenicity

Species:	rat Sex: female
Strain:	Sprague-Dawley
Route of admin.:	gavage
Exposure period:	10 days
Frequency of treatment:	Dosed on days 6-15 gestation
Duration of test:	
Doses:	0, 20, 70, 200 mg test material in 5 ml corn oil/kg
Control Group:	yes, concurrent vehicle
NOAEL Maternalt.:	70 mg/kg bw
NOAEL Teratogen.:	<= 200 mg/kg bw
NOAEL Fetal:	70 mg/kg bw
Method:	OECD Guide-line 414 "Teratogenicity"

Preliminary trials in 8 rats/group indicated that 600 mg/kg was lethal to 50% of maternal rats while 200 mg.kg caused decreased body weights in maternal and fetal animals. There were no effects at 20 or 70 mg/kg. Consequently, 200 mg/kg was selected as the top (high) dose in the definitive study, Confirmation of the test dose solutions were confirmed analytically.

The definitive study used 25 inseminated female rats per test group (0, 20, 70, and 200 mg of test substance/kg doses in five (5) mL corn oil/kg). The animals were dosed on Days 6-15 gestation. Body weights, food consumption, liver weights, clinical changes, pregnancy rates, and corpora lutea counts were followed along with numerous fetal parameters. All fetuses were weighed, sexed, and assessed for external and visceral abnormalities. One (1) half of the fetuses were examined for skeletal abnormalities while the second half were subjected to cranial bone assessments.

Remark: Administered in 5 ml corn oil/kg by gavage

Result: The test substance induced no lethality. Deficits were seen in maternal body weights (Day-12 and body weight change from Day-6 to Day-15) and food consumption (during treatment period) at the highest dose only (200 mg/kg). Pregnancy rates, litter sizes, number of live fetuses, uterine implantation, and all gestational parameters were unaffected by chemical treatment. There was a linear trend towards lower body weights in fetuses with increasing doses (approximately 5% decrease in 200 mg/kg group). Assessment of cranial, skeletal, visceral, and external appearance discerned no compound-related abnormalities (malformatiuons or variations) according to established criteria. The test material produced minimal effects (body weight) to maternal rats from oral dosing of 200 mg/kg during pregnancy. There was no induction by the test chemical of birth defects (major or minor) in fetal animals.

Year: 1995 GLP: yes
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (1) valid without restriction
08-AUG-2000

(22)

Species: rat Sex: male/female
Strain: Sprague-Dawley
Route of admin.: oral feed
Exposure period: Varied, see method
Frequency of treatment: Varied, see method
Duration of test:
Doses: 2500 ppm
Control Group: yes, concurrent vehicle
Method: other: Mechanistic Study

The toxicity of the test substance to maternal and 1st generation offspring was evaluated by exposing CD (Sprague-Dawley) rats to fixed dietary concentrations of 2500 ppm during different time periods (i.e. exposures during prebreed, mating, gestation, and/or lactation). Five (5) Groups (20/sex/Group) were studied including: Group one (1)- Negative control; Group two (2)- Dietary test substance during prebreed and mating, exposures ended on gestation day (gd)-0; Group three (3)- Dietary test substance during gestation and lactation, exposures began on gd-0; Group four (4)- Dietary test substance during prebreed, mating, gestation, and lactation, the Positive control and; Group five (5)- Dietary test substance during prebreed, mating, gestation, and lactation, plus 600 ppm of iron gluconate in the drinking water for prebreed through lactation.

Males and females were paired within Groups (1:1) for the two-week mating period. Once a given female was found to be sperm positive {date designated as gestation day (gd)-0}, "her" male was euthanized and discarded. On the day of delivery (pnd-0), pups were counted, sexed, and weighed. On pnd-4, litters were culled to ten, counted, sexed, and weighed. On pnd-7, -14, and -21, pups were counted, sexed, and weighed. All pups were euthanized and one (1)/sex/litter necropsied on pnd-21. Dead pups on pnd-0 and -1 were examined macroscopically (necropsied) for polycystic kidneys. Female body weights and feed consumption were recorded weekly during prebreed, gestation, and postnatally. At necropsy on pnd-21, the maternal spleen, liver, and kidneys were weighed and retained in a fixative. Kidneys form Groups one (1) and five (5) were examined histopathologically.

Blood sampling was performed ongestation day-21 and pnd-21 from all females (pregnant) by tail vein withdrawal. Blood sampling was performed on pnd-21 on the F1 offspring by withdrawal from the abdominal vena cava at sacrifice. The blood parameters assessed were: WBC, RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, Platelets, WBC Differential (to correct the RBC and WBC counts for Nucleated Red Blood Cells) and Methemoglobin. On gd-21, a second sample of blood was taken via tail vein from all pregnant females in all Groups, with plasma frozen for possible subsequent analysis for specific hormones. For Group three(3), any female who had not yet delivered by gestation day-23 had blood taken from the tail vein and plasma frozen. On pnd-21, the spleen, liver, kidneys, and heart from one(1) pup/sex/litter were weighed and retained in a fixative.The kidneys from all offspring were examined histologically. Statistical analysis included both parametric and nonparametric tests for continuous and discrete data.

Remark: The objectives of this study were to confirm and further characterize previously-observed effects following the test substance administration to pregnant rats. This study was designed (1) to determine the necessary and sufficient timing of exposure to maternal females at a fixed dietary concentration of the test substance to produce dystocia, prolonged gestation, and polycystic kidneys in offspring, (2) to determine whether the test substance results in demonstratable macrocytic anemia in maternal animals, (3) to determine if there is treatment-induced anemia and whether iron supplementation ameliorates or prevents the anemia, dystocia, and/or polycystic kidneys, and (4) to determine if F0 parental females exhibit polycystic kidneys due to dietary exposure to the test substance.

Result: F0 Males: The test substance intake over the prebreed period (Study Days 0-28) averaged 180 mg/kg/day for all three (3) exposed Groups {two (2), four (4), and five (5)}. Iron gluconate intake in Group five (5) averaged 56 mg/kg/day (Study Days-0 to 28). Clinical observations were found to be unrelated to compound administration.

F0 Females: The test substance intake averaged 187-192 mg/kg/day for Groups two (2), four (4) and five (5) during gestation days (gd)-0 to 28. Iron gluconate intake during gestational days-0 to 28 in Group five (5) averaged 53 mg/kg/day. Clinical observations during gestation included one (1) female found dead in Groups three (3) and four (4), alopecia predominantly in Groups four (4) and five (5), pale eyes and tail, pale (not otherwise specified) almost exclusively in Groups three(3), four (4) and five (5) (all exposed), pilorection in Groups three (3), four (4) and five (5), and delayed parturition in Groups three (3), four (4), and five (5).

The hematological profile of maternal rats on gestation day-21 found no evidence on macrocytic anemia in any groups.

REPRODUCTIVE/DEVELOPMENTAL: Gestational index (a measure of live litters relative to pregnant females) was significantly increased in Groups three (3) and four (4) but not in Group five (5). Male mating, fertility, and pregnancy indices were equivalent across all groups. Gestational length in days was significantly prolonged in Group three (3) (23.6+/-0.2), Group four (4) (23.8+/-0.2), and Group five (5) (23.5+/-0.2) relative to Control Group value (22.2+/-0.1) and the value in Group two (2) (22.3+/-0.1). Number of implantation sites per litter was significantly reduced in Group five (5). Percent of post implantation loss was significantly increased in Groups three (3) and four (4). Pups per litter were significantly reduced in Groups three (3), four (4) and five (5), and number of dead pups per litter were significantly increased in Groups three (3) and four (4). Weanling gross and microscopic findings were limited to hydronephrosis in Groups one (1) and two (2), gas in intestines in Group two (2), and gross evidence of polycystic kidneys in Groups three (3), four (4), and five (5). Maternal hematologic profiles at sacrifice (21 days after delivery) indicated statistically significant changes in most erythrocyte parameters. The white blood cell differential counts indicated changes (as percent of cells examined) as follows: increase in segmented neutrophils and decrease in lymphocytes only in Group four (4), with no treatment-related changes in the percentages of monocytes or eosinophils. Histopathologic assessment was performed on kidneys of all maternal rats in Groups one (1) and five (5). Polycystic kidneys were observed microscopically (but not macroscopically) in three (3) of 20 animals in Group five (5), with no polycystic kidneys observed in Group one (1).

The timing of exposure to the test substance with respect to pregnancy is an important determinant of toxicity. Exposure of F0 females to 2500 ppm of the test material during gestation is necessary and sufficient to produce dystocia (prolonged gestation).

It is necessary and sufficient to expose F0 dams during gestation and/or lactation to produce polycystic kidneys in the F1 offspring. Since no Groups were exposed only during gestation or only during lactation, it is not possible to further define how exposure timing affects this endpoint. There was no demonstrable macrocytic anemia in gestation day-21 (gd-21) F0 dams in any treatment Group, but at post delivery day-21 (pnd-21), F0 mothers exposed prior to and during mating, gestation, and lactation were anemic. The F1 offspring at pnd-21 did not consistently display evidence of macrocytic anemia. Iron supplementation did not affect pnd-21 maternal anemia, dystocia, or incidence/severity of polycystic kidneys in the F1 offspring. However, perinatal survival of the offspring was affected. Microscopic, but not macroscopic evidence of polycystic kidneys was found in 15 percent of dams treated prior to and during mating, gestation, and lactation (with iron supplementation). Controls had neither macroscopic nor microscopic indications of polycystic kidneys. Exposure of animals to the test substance prior to and during mating {Group two (2)} did not appear to result in adverse affects to offspring. Furthermore, exposure during the prebreed/mating periods did not increase the affects produced from gestation/lactation exposures only.

Year: 2000 GLP: no
Test substance: Wingstay 100 (mixed diaryl-p-phenylenediamines)

Reliability: (2) valid with restrictions
Although this study was not conducted to GLP, the test parameters used were based on a sound scientific design.
09-AUG-2000

(15)

5.10 Other Relevant Information

Type: other: A Photoirritation Study in Rabbits
Method: US FDA test guidelines and GLPs.
Result: UV light did not enhance the skin irritation response of the test substance in rabbits, and therefore is not considered to be a photo-irritant.

Test condition: Albino rabbits (4 females,4 males) were shaved in the dorsal portion of the animals trunk. One day later, 0.5 g of test material was placed onto 2 skin site of 3 male and 3 female rabbits. 0.5 ml of Oxsoralen lotion was similarly applied to 1 male and 1 female rabbit. After 2-hour skin contact exposure period, the gauze patches were removed from the animals' right sides and the left side sites were covered with aluminum foil to prevent light exposure. All animals were exposed to UVA light for 40 minutes. Following light exposures, the gauze patches were reattached for additional 21 hours.

Skin sites were scored according to Draize procedures at 25, 48 and 72 hours plus 7 days following cessation of chemical exposure.

Reliability: (1) valid without restriction

(1)

other: Mechanistic

Method: Dietary WINGSTAY 100 (mixed diaryl-p-phenylenediamines) induced dystocia and delayed parturition with associated maternal deaths in pregnant rats in a 2-generation reproduction study. This mechanistic study was designed to assess exposure conditions necessary to induce these findings, and the role of possible iron deficiency. Female rats were exposed to 2500 ppm of WINGSTAY 100 in the diet as follows:

Group 1- 0 ppm for 12 week study (negative control)
Group 2- Exposed 4 weeks prebreed plus 2 weeks mating
Group 3- Exposed 3 weeks gestation plus 3 weeks lactation
Group 4- Exposed 4 weeks prebreed, 2 weeks mating, 3 weeks gestation, 3 weeks lactation (positive control)
Group 5- Positive control plus iron supplementation (600 ppm iron gluconate in drinking water)

Females (20/group) were mated with males with comparable dietary exposures. Following confirmed mating, males were sacrificed without further assessment. Rats were subjected to daily observations, weekly Body Weights (BWs), and feed and water consumptions. Maternal F0 rats were bled on gestational day 21 prior to delivery and post delivery day 21. A sample of plasma was frozen from the gestation day 21 bleeding for possible future endrocrine assessments. F1 rats were bled on day 21 post natal. Samples were subjected to standard hematology and metHgb assays. Major organ weights were determined. Observations were made during reproductive, gestational, and postnatal periods of the study. Necropsies with organ weights determinations were performed on all surviving F0 and F1 rats 21 days post delivery. Microscopic exams were performed on gross lesions in F0 rats, and on kidneys of F0 and F1 animals.

Remark: The study confirmed results in a 2-generation reproduction rat study that demonstrated dietary WINGSTAY 100 induces dystocia, delayed parturitition, and an associated decrease in pup survival at birth.

These findings have earlier been associated with DPPD and DPA according to available literature. The effects in Group 3, but not Group 2 indicate that chemical exposure during gestational period is essential for the dystocia and delayed parturition observed. Since Group 3 included exposure during lactation, it is uncertain whether gestational exposure alone would induce the polycyctic kidneys in offspring. Pre-gestational exposure did not enhance the effects attributed to gestational WINGSTAY 100 ingestion. Finally, although iron supplementation had no apparent impact on blood parameters, it did decrease the number of stillbirths without impacting other reproductive or litter endpoints.

Result: Body weights and feed consumption for F0 rats were reduced relative to negative controls, possibly as a result of decreased palatability of the WINGSTAY 100-containing diet. One (1) Group 3 female died on gestation day 19, and one (1) Group 4 rat on gestation day 24. Due to dead litters, additional Groups 3 and 4 dams were euthanized. Other clinical observations included alopecia and pale appearance (eyes, tails and ears) in Groups 2-5 throughout study. There were no indications of RBC, WBC, or Hgb changes ascribed to WINGSTAY 100 exposure. RBC size distribution width was decreased, demonstrating lack of macrocytic changes. The fertility indices (number of pregnancies/number of matings) were 79, 74, 90, 79, and 71%. Gestational indices (number of females with live litters/number of pregnancies) were 100, 93, 65, 71, and 100%, and the gestational lengths were 22.2, 22.3, 23.6, 23.8, and 23.5 days (Groups 3-5 were significantly delayed). Litter effects included stillbirths (3, 1, 45, 46, and 10% of total pups delivered), decreased pup survival (13, 13, 6, 7, and 8 live pups/litter) on post natal day 0 and 10, 10, 6, 8, and 7 on day 21. Relative liver and heart weights were increased for Groups 3-5 F1 pups. Gross observations included polycyctic kidneys in male and female F1 Groups 3-5 pups, confirmed microscopically in part as dilatation in the papillary region. Rates of these renal lesions were in excess of 80% in both male and female rats. Microscopic results for the F0 females included a 15% incidence of polycyctic kidneys in Group 5 and none in Group 1. The other groups were not examined microscopically.

Date: 2/7/00
Test Substance: Wingstay 100 (mixed diaryl-p-phenylenediamines

Reliability: (1) valid without restriction

(14)

(1) A Photoirritation Study in Rabbits with WINGSTAY 100, Report # 3097.28 to The Goodyear Tire & Rubber Company, August 24, 1995

(2) 38-Week Accelerated Bioassay (ABA) of WINGSTAY 100 in Rats, American Health Foundation, 1996

(3) 52-Week Chronic Feeding Study of WINGSTAY 100 in Rats with 12-Week Recovery. Report # AHF R1695, American Health Foundation, 12/16/1996

(4) American Health Foundation, 32P Postlabeling Assay for Detection of Adduct Formation by WINGSTAY 100 and R-59 in Rat Liver and Urinary Bladder DNA, AHF Study No. 93-63 to The Goodyear Tire & Rubber Company, 1995

(5) American Health Foundation, Preliminary Oral 3-Week Range-Finding Study for Administration of WINGSTAY 100 and R-59 in Male and Female Fischer 344 Rats, AHF Study R-1626 to The Goodyear Tire & Rubber Company, 1994.

(6) Bayer AG Data

(7) Bayer AG, Report No. 19778, December 10, 1990.

(8) Bayer AG, Report Number 19940, February 4, 1991.

(9) Bayer AG, Unpublished Data, July 2, 1992

(10) Bioaccumulation Test of Wingstay 100 in Carp, Report Study Number 43172, Kurume Research Laboratories/CITI, 12/18/1998

(11) Four-Week Dietary Study of WINGSTAY 100 in Fischer 344 Rats, Report # AHF R1664, American Health Foundation, 1/31/96

(12) Litton Bionetics, Inc., Balb/3T3 In Vitro Transformation Assay of NAILAX, Genetics Assay No.5419 to The Goodyear Tire & Rubber Company, 1981.

(13) Litton Bionetics, Inc., Drosophilia SLRL Assay with Nailax to The Goodyear Tire & Rubber Company, 1979.

(14) Mechanistic Study of WINGSTAY 100, Report #: 65C-6429-500, Research Triangle Institute, 2/7/00

(15) Mechanistic Study of Wingstay 100, Report Study # RTI 65C-6429-500, Research Triangle Park, February 11, 2000

(16) Pharmakon USA, Report # Ph301-GY-001-93 to The Goodyear Tire & Rubber Company, 1993

(17) Pharmakon USA, Report # Ph301-GY-004-93 to The Goodyear Tire & Rubber Company, 1994

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